

## Regulation of Proteins Implicated in Alzheimer's Disease by MicroRNAs.

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the deposition of Amyloid-Beta (A $\beta$ ) peptide in the brain. This toxic peptide is generated by the sequential cleavage of Amyloid Precursor Protein (APP) by Beta-site APP-cleaving enzyme-1 (BACE-1) and  $\gamma$ -secretase. The disorder is also characterized by the perturbation of calcium homeostasis in neurons. MicroRNAs are short, single-stranded RNAs that are able to influence protein expression by targeting the 3' Untranslated region (UTR) or 5' UTR of mRNAs. Previous work in our laboratory has shown that miR-101, miR-153 and miR-346 can regulate APP whereas miR-339-5p can lower BACE1 expression. Here, we aim to reduce APP, BACE1 and A $\beta$  levels, in vitro, by the addition of microRNAs that target the 3' UTR of APP and BACE1. We show that in a human astrocytoma-glioblastoma (U373) cell line, the expression of BACE1 protein is significantly reduced compared to the mock condition upon transfecting miR-298, miR-328 and miR-144. miR-298 also reduces A $\beta$  levels in these cells. Similarly, in HeLa cells, we show that miR-520c, miR-20b and miR-144 produce a reduction in APP expression compared to both mock and a negative control microRNA mimic. Additionally, we observed that knocking down APP using siRNA, but not knocking down BACE1, lowers basal intracellular calcium levels as well as changes the kinetics of Potassium Chloride (KCl)-induced intracellular calcium influx in a human fetal brain (HFB) culture, when compared to control. miR-346 increases basal calcium levels, but does not affect KCl-induced calcium transients in our HFB culture. Taken together, these results show that miRNAs can influence both the protein expression as well as calcium homeostasis in different human cell culture models. By reducing levels of proteins implicated in AD pathology and by reversing calcium dysregulation, our results will benefit AD research and generate possibilities for novel therapeutics.

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